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(57) Abstract

An effective anti-IL-5 recombinant antibody molecule comprising heavy and/or light chain antigen-binding residues from a donor antibody.

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5
   GAA TCT GGA GGA GGC TTG GTA CAG CCA TCA CAG ACC CTG TCT CTC
    E  S  G  G  G  L  V  Q  P  S  Q  T  L  S  L>

   ACC TGC ACT GTC TCT GGG TTA TCA TTA ACC AGC AAT AGT GTG AAC
10  T  C  T  V  S  G  L  S  L  T  S  N  S  V  N>

   TGG ATT CGG CAG CCT CCA GGA AAG GGT CTG GAG TGG ATG GGA CTA
    W  I  R  Q  P  P  G  K  G  L  E  W  M  G  L>

15 ATA TGG AGT AAT GGA GAC ACA GAT TAT AAT TCA GCT ATC AAA TCC
    I  W  S  N  G  D  T  D  Y  N  S  A  I  K  S>

   CGA CTG AGC ATC AGT AGG GAC ACC TCG AAG AGC CAG GTT TTC TTA
    R  L  S  I  S  R  D  T  S  K  S  Q  V  F  L
20
   AAG ATG AAC AGT CTG CAA AGT GAA GAC ACA GCC ATG TAC TTC TGT
    K  M  N  S  L  Q  S  E  D  T  A  M  Y  F  C>

   GCC AGA GAG TAC TAC GGC TAC TTT GAT TAC TGG GGC CAA GGA GTC
25  A  R  E  Y  Y  G  Y  F  D  Y  W  G  Q  G  V

   ATG GTC ACA GTC TCC TCA
    M  V  T  V  S  S>
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INTERLEUKIN-5 SPECIFIC RECOMBINANT ANTIBODIES

The present invention relates to a recombinant antibody molecule (RAM), and especially a humanized antibody molecule (HAM) having specificity for human interleukin-5 (hIL-5), the nucleic acids which encode the heavy and light chain variable domains of said recombinant antibody, a process for producing said antibody using recombinant DNA technology and the therapeutic use of the recombinant antibody.

In the present application, the term "recombinant antibody molecule" (RAM) is used to describe an antibody produced by a process involving the use of recombinant DNA technology. The term "humanized antibody molecule" (HAM) is used to describe a molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable domains fused onto constant domains or one of more complementary determining regions (CDRs) grafted onto appropriate framework regions in the variable domain. The abbreviation "MAb" is used to indicate a monoclonal antibody.

The term "recombinant antibody molecule" includes not only complete immunoglobulin molecules but also any antigen binding immunoglobulin fragments, such as Fv, Fab and $F(ab')_2$ fragments, and any derivatives thereof, such as single chain Fv fragments.

Natural immunoglobulins have been used in assay, diagnosis and, to a limited extent, therapy. The use of immunoglobulins in therapy has been hindered as most antibodies of potential use as therapeutic agents are MAbs produced by fusions of a rodent spleen cells with rodent myeloma cells. These MAbs are therefore essentially rodent proteins. The use of these MAbs as therapeutic agents in human can give rise to an undesirable immune response termed the HAMA (Human Anti-mouse Antibody) response. The use of rodent MAbs as therapeutic agents in humans is inherently

limited by the fact that the human subject will mount an immunological response to the MAb which would either remove it entirely or at least reduce its effectiveness.

A number of techniques to reduce the antigenic characteristics of such non-human MAbs have been developed. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule. These methods are generally termed "humanization" techniques.

Early methods for humanizing MAbs involved the production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody are fused to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP 0120694 (Celltech Limited) and EP 0125023 (Genentech Inc. and City of Hope). Humanized chimeric antibodies, however, still contain a significant portion of non-human amino acid sequences, and can still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al., Br. J. Cancer, 62, 487 (1990)].

An alternative approach, described in EP-A-0239400 (Winter), involves the grafting of the complementarity determining region (CDRs) of a mouse MAb on to framework regions of the variable domains of a human immunoglobulin using recombinant DNA techniques. There are three CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains. Such CDR-grafted humanized antibodies are much less likely to give rise to a HAMA response than humanized chimeric antibodies in view of the much lower proportion of non-human amino acid sequences which they contain. In Riechmann et al. [Nature, 332 323-324 (1988)] it was found that the transfer of the CDRs alone, as defined by Kabat [Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (1987)], was not sufficient to provide satisfactory antigen binding activity

in the CDR-grafted product. Riechmann et al. found that it was necessary to convert a number of residues outside the CDRs, in particular in the loop adjacent CDR1. However, the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than that of the original MAb.

In WO 91/09967, Adair et al. described CDR-grafted antibody heavy and light chains, and determined a hierarchy of donor residues.

In WO 93/16184, Chou et al. described the design, cloning and expression of humanized monoclonal antibodies against human interleukin-5. A method for selecting human antibody sequences to be used as human frameworks for humanization of an animal antibody is suggested, comprising the steps of comparing human variable domain sequences with the variable domain sequences of the animal MAb that is to be humanized for percentage identities, sequence ambiguities and similar PIN-region spacing. PIN-region spacing is defined as the number of residues between the cysteine residues forming the intra domain disulfide bridges. The human antibody having the best combination of these features is selected. A method for determining which variable domain residues of an animal MAb which should be selected for humanization is also suggested, comprising determining potential minimum residues (residues which comprise CDR structural loops and the residues required to support and/or orientate the CDR structural loops) and maximum residues (residues which comprise Kabat CDRs, CDR structural loops, residues required to support and/or orientate the CDR structural loops and residues which fall within about 10 Å of a CDR structural loop and possess a water solvent accessible surface of about 5 $Å^2$ or greater) of the animal monoclonal antibody. Furthermore, computer modelling is performed on all possible recombinant antibodies, comprising the human antibody framework sequence into which minimum and maximum residues have been inserted. The minimum or maximum residues are selected based on the combination which produces a

recombinant antibody having a computer-model structure closest to that of the animal monoclonal antibody. The humanized anti-IL-5 antibody obtained appears to have lost a substantial amount of its affinity for the hIL-5 molecule.

It is an aim of the present invention to provide a humanized antibody molecule having improved affinity for the hIL-5 molecule.

Accordingly the present invention provides a RAM having affinity for human IL-5 and comprising antigen binding regions derived from heavy and/or light chain variable domains of a donor antibody having affinity for human IL-5, the RAM having a binding affinity similar to that of the donor antibody.

The RAM of invention may comprise antigen binding regions from any suitable donor anti-IL-5 antibody. Typically the donor anti-IL-5 antibody is a rodent MAb. Preferably the donor antibody is MAb 39D10.

The variable domains of the heavy and light chains of MAb 39D10 are hereinafter specifically described with reference to Figures 1 and 2.

According to one preferred aspect of the invention, the RAM of the present invention is an anti-IL-5 antibody molecule having affinity for the human IL-5 antigen comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising predominantly acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for human IL-5, wherein said composite heavy chain comprises donor residues at least at positions 31-35, 50-65 and 95-102 (according to the Kabat numbering system) [Kabat *et al.*, Sequences of Proteins of Immunological Interest, Vol I, Fifth Edition, 1991, US Department of Health and Human

Services, National Institute of Health].

Preferably, the composite heavy chain framework additionally comprises donor residues at positions 23, 24, 27-30, 37, 49, 73 and 76-78 or 24, 27-30, 37, 49, 73, 76 and 78.

According to a second preferred aspect of the present invention, there is provided an anti-IL-5 antibody molecule having affinity for a human IL-5 antigen comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising predominantly acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for human IL-5, wherein said composite light chain comprises donor residues at least at positions 24-34, 50-56 and 89-97 (according to the Kabat numbering system).

Preferably, the composite light chain framework additionally comprises donor residues at positions 22, 68 and 71 or at positions 68 and 71.

According to a third preferred aspect of the present invention, there is provided an anti-IL-5 antibody molecule having affinity for a human IL-5 antigen comprising a composite heavy chain according to the first aspect of the invention and a composite light chain according to the second aspect of the invention.

Preferably, each RAM of the invention has an affinity constant for human IL-5 of greater than 10^{-9}M.

It will be appreciated that the invention is widely applicable to the production of anti-IL-5 RAMs in general. Thus, the donor antibody may be any anti-IL-5 antibody derived from any animal. The acceptor antibody may be derived from an animal of the same species and may even be of the same antibody class or sub-class. More usually,

however, the donor and acceptor antibodies are derived from animals from different species. Typically, the donor anti-IL-5 antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

Any appropriate acceptor variable framework sequence may be used having regard to class or type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same or similar class or type as that of the donor antibody. Conveniently, the framework chosen has the most homology to the donor antibody. Preferably, the human group III gamma germ line frameworks are used for the composite heavy chain and the human group I kappa germ line frameworks are used for the composite light chains.

The constant region domains of the RAMs of the invention may be selected having regard to the proposed functions of the antibody, in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and the IgG3 isotype, when the humanized antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used where the humanized antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for specifically binding to and neutralizing the biological activity of human IL-5. Modified human constant region domains may also be used in which one or more amino acid residues have been altered or deleted to change a particular effector function. Preferably, the constant region domains of the RAMs are human IgG4.

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [Kabat *et al.*, Sequences of Proteins of

Immunological Interest, Vol I, Fifth Edition, 1991, US Department of Health and Human Services, National Institute of Health]. Thus, the residue designations do not always correspond directly with a linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the Kabat numbering, corresponding to a shortening of, or insertion into, the basic variable domain structure.

Also the anti-IL-5 antibody molecules of the present invention may have attached to them effector or reporter molecules. Alternatively, the procedures of recombinant DNA technology may be used to produce immunoglobulin molecules in which the Fc fragment or CH3 domain of a complete immunoglobulin has been replaced by, or has been attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme, cytokine, growth factor or toxin molecule.

Thus, the remainder of the antibody molecules need not comprise only sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

Further aspects of the invention include DNA sequences coding for the composite heavy chain and the composite light chain. The cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences and the processes for producing the antibody molecules comprising expressing the DNA sequences in the transformed host cells are also further aspects of the invention.

The general methods by which vectors may be constructed, transfection methods and culture methods are well known in the art and form no part of the invention.

The DNA sequences which encode the anti-IL-5 donor amino acid sequences may be obtained by methods well known in the art (see, for example, International Patent Application No. WO 93/16184). For example, the anti-IL-5 coding sequences may be obtained by genomic cloning or cDNA cloning from suitable hybridoma cell lines, e.g. the 39D10 cell line. Positive clones may be screened using appropriate probes for the heavy and light chains required. Also PCR cloning may be used.

The DNA coding for acceptor amino acid sequences may be obtained in any appropriate way. For example, DNA sequences coding for preferred human acceptor frameworks such as human group I light chains and human group III heavy chains, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare the desired DNA sequences. The sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example, oligonucleotide directed synthesis as described by Jones et al. [Nature, 321, 522 (1986)] may be used. Also oligonucleotide directed mutagenesis of a pre-existing variable region as, for example, described by Verhoeyen et al. [Science, 239, 1534-1536 (1988)] may be used. Also enzymatic filling in of gapped oligonucleotides using T4 DNA polymerase as, for example, described by Queen et al. [Proc. Natl. Acad. Sci. USA, 86, 10029-10033 (1989) and WO 90/07861] may be used.

Any suitable host cell and vector system may be used for the expression of DNA sequences coding for the RAM. Preferably, eucaryotic, e.g. mammalian, host cell expression systems are used. In particular, suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, according to a further aspect of the present invention

a process for producing an anti-IL-5 RAM is provided comprising:

(a) producing in a first expression vector a first operon having a DNA sequence which encodes a composite heavy chain, as defined according to the first preferred aspect of the invention;

(b) optionally producing in the first or a second expression vector a second operon having a DNA sequence which encodes a complementary light chain, which may be a composite light chain as defined according to the second preferred aspect of the invention;

(c) transfecting a host cell with the or each vector;

and

(d) culturing a transfected cell line to produce the RAM.

Alternatively, the process may involve the use of sequences encoding a composite light chain and a complementary heavy chain.

For the production of RAMs comprising both heavy and light chains, the cell lines may be transfected with two vectors. The first vector may contain an operon encoding a composite or complementary heavy chain and the second vector may contain an operon encoding a complementary or composite light chain. Preferably, the vectors are identical except insofar as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed. In a preferred alternative, a single vector may be used, the vector including the sequences encoding both the heavy chain and the light chain.

The DNA in the coding sequences for the heavy and light

chains may comprise cDNA or genomic DNA or both.

The present invention also includes therapeutic and diagnostic compositions comprising the RAMs and uses of such compositions in therapy and diagnosis.

Accordingly, in a further aspect the invention provides a therapeutic or diagnostic composition comprising a RAM according to previous aspects of the invention in combination with a pharmaceutically acceptable excipient, diluent or carrier.

These compositions can be prepared using the RAMs of the present invention, for instance as whole antibodies, single chain Fv fragments or antibody fragments, such as Fab or Fv fragments. Such compositions have IL-5 blocking or antagonistic effects and can be used to suppress IL-5 activity.

The compositions according to the invention may be formulated in accordance with conventional practice for administration by any suitable route, and may generally be in a liquid form [e.g. a solution of the RAM in a sterile physiologically acceptable buffer] for administration by for example an intravenous, intraperitoneal or intramuscular route; in spray form, for example for administration by a nasal or buccal route; or in a form suitable for implantation.

The invention also provides a method of therapy or diagnosis comprising administering an effective amount, preferably 0.1 to 10 mg/kg body weight, of a RAM according to previous aspects of the invention to a human or animal subject. The exact dosage and total dose will vary according to the intended use of the RAM and on the age and condition of the patient to be treated. The RAM may be administered as a single dose, or in a continuous manner over a period of time. Doses may be repeated as appropriate.

The RAM according to previous aspects of the invention may be used for any of the therapeutic uses for which anti-IL-5 antibodies, e.g. 39D10, have been used or may be used in the future.

IL-5 is a primary activator of eosinophils, and blocking the function of this cytokine with antibodies has been shown to prevent or reduce eosinophilia which is associated with certain allergic diseases. Thus the RAM according to the invention may be used for this purpose, and in particular may be of use in the treatment of asthma, where it may be expected to prevent the accumulation and activation of eosinophils in asthmatic lungs, thereby reducing bronchial inflammation and airway narrowing. For use in the treatment of asthma the RAM according to the invention may advantageously be a single chain Fv fragment, formulated as a spray, for administration for example via the nasal route.

A preferred protocol for obtaining an anti-IL-5 antibody molecule in accordance with the present invention is set out below. This protocol is given without prejudice to the generality of the invention as hereinbefore described and defined.

The 39D10 rat monoclonal antibody raised against human IL-5 is used as the donor antibody. The variable domains of the heavy and light chains of 39D10 have previously been cloned (WO 93/16184) and the nucleotide and predicted amino acid sequences of these domains are shown in Figures 1 and 2. The appropriate acceptor heavy and light chain variable domains must be determined and the amino acid sequence known. The RAM is then designed starting from the basis of the acceptor sequence.

1. <u>The CDRs</u>

At a first step, donor residues are substituted for acceptor residues in the CDRs. For this purpose, the CDRs are preferably defined as follows:

heavy chain:	CDR1:	residues 31-35
	CDR2:	residues 50-65
	CDR3:	residues 95-102
light chain:	CDR1:	residues 24-34
	CDR2:	residues 50 to 56
	CDR3:	residues 89 to 97

The positions at which donor residues are to be substituted for acceptor residues in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. HEAVY CHAIN

2.1 Donor residues are used either at all of positions 24, 27 to 30, 37, 49, 73, 76 and 78 or at all of positions 23, 24, 27 to 30, 37, 49, 73 and 76 to 78 of the heavy chain.

3. LIGHT CHAIN

3.1 Donor residues are used either at all of positions 22, 68 and 71 or at all of positions 68 and 71.

The present invention relates to a recombinant anti-IL-5 antibody molecule having a binding affinity substantially equal to that of the donor antibody. The present invention is now described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows the nucleotide and amino acid sequence of the 39D10 heavy chain;

Figure 2 shows the nucleotide and amino acid sequence of

the 39D10 light chain;

Figure 3 shows the alignment of the 39D10 heavy chain variable domain framework regions with the heavy chain variable domain framework regions of the consensus sequence of the human group III heavy chains;

Figure 4 shows the alignment of the 39D10 light chain variable domain framework regions with the light chain variable domain framework regions of the consensus sequence of the human group I light chains;

Figure 5 shows the nucleotide and amino acid sequence of the CDR grafted anti-IL-5 light chain CTIL-5-gL6;

Figure 6 shows the nucleotide and amino acid sequence of the CDR grafted anti-IL-5 heavy chain CTIL-5-10gH;

Figure 7 shows a map of plasmid pMR14;

Figure 8 shows a map of plasmid pMR15.1;

Figure 9 shows the affinity constants and association and disassociation rates of a chimeric 39D10 antibody and the CTIL-5-10gH\-gL6 antibody;

Figure 10 shows a graph of the neutralisation of IL-5 in the TF1 assay by a panel of antibodies;

Figure 11 shows the results of a competition assay for rat 39D10, a chimeric 39D10 antibody and the CTIL-5-10gH/gL6 antibody; and

Figure 12 shows the effect of CTIL-5-10gH/gL6 on monkey

eosinophilia.

EXAMPLE

1. MATERIAL AND METHODS

39D10 is a rat monoclonal antibody raised against human IL-5. The genes for the variable domains of the heavy and light chains of 39D10 have previously been cloned (WO 93/16184) and the nucleotide and predicted amino acid sequences of these domains are shown in Figures 1 and 2. Because of the strategy used in the cloning of the variable domain of the 39D10 heavy chain, the first five amino acids of the framework regions are unknown. However, a heavy chain was available which contained the leader sequence and the first five amino acids of framework 1 from the antibody YTH 34.5HL, Riechmann et al., [Nature, 332, 323-327 (1988)].

2. MOLECULAR BIOLOGY PROCEDURES

The molecular biology procedures used were as described in Maniatis et al. [Molecular Cloning: A Laboratory Manual, Second Edition, Vols 1 to 3, Cold Spring Harbor Laboratory Press (1989)].

3. CONSTRUCTION OF RECOMBINANT HEAVY AND LIGHT CHAIN GENES

Heavy Chain

A heavy chain Vh region was generated by PCR using the oligonucleotides R3601 and R2155. The sequences of these are:

R3601 5'GCGCGCAAGCTTGCCGCCACCATGAAG(A,T)TGTGGTTAAACTGGGTTTT3'

R2155 5'GCAGATGGGCCCTTCGTTGAGGCTG(A,C)(A,G)GAGAC(G,T,A)GTGA3'

The reaction mixture (100µl) contained 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM of

each deoxyribonucleoside triphosphate, 0.1 μg 39D10 heavy chain DNA, 6 pmoles of R3601 and R2155 and 0.25 units Taq polymerase. The reaction mixture was heated at 94°C for 5 minutes and then cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, the reaction was extracted with an equal volume of phenol/chloroform (1:1 v/v), then with chloroform before being precipitated by the addition of 2.5 volumes of ethanol. The PCR product was dissolved in the appropriate buffer, digested with HindIII and ApaI, purified on an agarose gel and ligated into the vector pMR14 (Figure 7) which had also been digested with HindIII and ApaI. Following transformation into *E. coli* LM1035, colonies were grown overnight and plasmid DNA analysed for Vh inserts. The nucleotide sequence of the Vh region in plasmid, pARH1217, is shown in Figure 1.

Light Chain

A Vl light chain gene was generated from the original Vl, as described in WO 93/16184, clone by PCR with the oligonucleotides R3585 and R3597. The sequences of these are:

R3585 5'GGACTGTTCGAAGCCGCCACCATGAGTGTGCTCACTCAGGTCCT3'
R3597 5'GGATACAGTTGGTGCAGCATCCGTACGTTT3'

PCR was carried out as described above. The PCR product was digested with the enzymes BstBI and SplI and, after purification, ligated into pMR15.1 (Figure 8) that had previously been digested with the same enzymes.

A colony was identified, after transformation of *E. coli* LM1035, that contained a plasmid (pARH1215) with a Vl insert. The nucleotide sequence of the Vl insert is shown in Figure 2.

CDR Grafting of 39D10

Light Chain

In order to decide on the most appropriate human acceptor frameworks for the CDR loops of 39D10, the amino acid sequence of frameworks 1-3 of 39D10 were compared with those of known human kappa light chains. 39D10 was found to be most homologous to human group I light chains. Based on this, it was decided to use the human group I germ line frameworks for the CDR grafting. The homologies between these sequences are shown in Figure 3. Also shown is the homology between the framework 4 regions of 39D10 and the consensus sequence of known human group I light chains. The residues in 39D10 that differ from the human consensus sequence are underlined. The contribution that these residues might make to antigen binding was analysed and two genes were constructed for the CDR grafted light chain. These were CTIL-5-gL5 and CTIL-5-gL6 in which, as well as the CDR residues, either residues 22, 68 and 71 or residues 68 and 71 were also from 39D10 respectively. The nucleotide and amino acid sequences of CTIL-5-gL6 are shown in Figure 5.

Heavy Chain

CDR grafting of the 39D10 heavy chain was carried out as described for the light chain. The framework regions of 39D10 were found to be most homologous to those of human group III antibodies and, consequently, the consensus sequence of the frameworks of the human group III germ line genes was used to accept the CDRs of the 39D10 heavy chain. As before, the consensus sequence for human group III framework 4 regions was also chosen. A comparison of these sequences is shown in Figure 4 with the residues in 39D10 that differ from the human consensus sequence underlined.

Analysis of the framework residues in 39D10 that might influence antigen binding was carried out and, based on

this, two genes, CTIL-5-9gH and CTIL-5-10gH, were constructed in which either residues 23, 24, 27 to 30, 37, 49, 73 and 76 to 78 or residues 24, 27-30, 37, 49, 73, 76 and 78 respectively were from 39D10. The nucleotide and amino acid sequences of CTIL-5-10gH is shown in Figure 6.

Expression and Bioactivity of Anti-IL-5 Antibodies

Chimeric (rat/human) and CDR grafted 39D10 were produced for biological evaluation by transient expression of the heavy and light chain pairs after co-transfection into Chinese Hamster Ovary (CHO) cells using calcium phosphate precipitation.

On the day prior to transfection, semi-confluent flasks of CHO-L761h cells (Cockett et al., Nucl. Acids. Res., 19, 319-325, 1991) were trypsinised, the cells counted and T75 flasks set up each with 10^7 cells. On the next day, the culture medium was changed 3 hours before transfection. For transfection, the calcium phosphate precipitate was prepared by mixing 1.25ml of 0.25M $CaCl_2$ containing 50 μg of each of heavy and light chain expression vectors with 1.25 ml of 2xHBS (16.36 g NaCl, 11.9 gm HEPES and 0.4 g Na_2HPO_4 in 1 litre water with the pH adjusted to 7.1 with NaOH) and adding immediately into the medium of the cells. After 3 hours at 37°C in a CO_2 incubator, the medium and precipitate were removed and the cells shocked by the addition of 15 ml 15% glycerol in phosphate buffered saline (PBS) for 1 minute. The glycerol was removed, the cells washed once with PBS and incubated for 48-96 hours in 25 ml medium containing 10 mM sodium butyrate. Antibody was purified from the culture medium by binding to and elution from protein A - Sepharose. Antibody concentration was determined using a human Ig ELISA (see below).

ELISA

Antibody expression was assessed by transfecting pairs of

heavy and light chain genes into CHO cells and, after three days incubation, measuring the amount of antibody accumulating in the culture medium by ELISA.

For the ELISA, Nunc ELISA plates were coated overnight at 4°C with a $F(ab')_2$ fragment of a polyclonal goat anti-human Fc fragment specific antibody (Jackson Immunoresearch, code 109-006-098) at 5 µg/ml in coating buffer (15mM sodium carbonate, 35mM sodium hydrogen carbonate, pH6.9). Uncoated antibody was removed by washing 5 times with distilled water. Samples and purified standards to be quantitated were diluted to approximately 1 µg/ml in conjugate buffer (0.1M Tris-HCl pH7.0, 0.1M NaCl, 0.2% v/v Tween 20, 0.2% w/v Hammersten casein). The samples were titrated in the microtitre wells in 2-fold dilutions to give a final volume of 0.1 ml in each well and the plates were incubated at room temperature for 1 hour with shaking. After the first incubation step, the plates were washed 10 times with distilled water and then incubated for 1 hour as before with 0.1 ml of a mouse monoclonal anti-human kappa (clone GD12) peroxidase conjugated antibody (The Binding Site, code MP135) at a dilution of 1 in 700 in conjugate buffer. The plate was washed again and substrate solution (0.1 ml) added to each well. Substrate solution contained 150 µl N,N,N,N-tetramethylbenzidine (10 mg/ml in DMSO), 150 µl hydrogen peroxide (30% solution) in 10 ml 0.1M sodium acetate/sodium citrate, pH6.0. The plate was developed for 5-10 minutes until the absorbence at 630nm was approximately 1.0 for the top standard. Absorbence at 630nm was measured using a plate reader and the concentration of the sample determined by comparing the titration curves with those of the standard.

Determination of Affinity Constants for Anti-IL-5 Antibodies

Affinities of the chimeric and CDR grafted anti-IL-5 antibodies were determined using Biospecific Interaction Analysis (BIA). Antibodies were produced in CHO cells by

transfection of combinations of heavy and light chain genes and purified from culture supernatants on Protein A Sepharose. For affinity measurements, a polyclonal anti-human Fc antibody was bound to the Pharmacia Biosensor chip (12150 relative response units, RU) and used to capture anti-IL-5 which was passed over the chip at 5 µg/ml in 10mM HEPES, 0.15M NaCl, 3.4mM EDTA, pH7.4. The amount of anti-IL-5 captured for each run was approximately 1600 RU. Recombinant human IL-5 was then passed over the Sensorchip at various concentrations (0.6 to 5 µg/ml) in the above buffer. The Sensorchip was cleaned after each run with 100mM HCl and 100mM orthophosphoric acid to remove bound IL-5 and antibody. The sensorgrams generated were analysed using the kinetics software available with the BIAcore machine.

Values for the affinity constants and association and dissociation rates of two antibodies, chimeric 39D10 and CTIL-5-10gH/-gL6, were determined. The results are shown in Figure 9. It can be seen that chimeric 39D10 has an extremely high affinity for human IL-5 and that this value has been reproduced in CTIL-5-10gH/-gL6.

Activity of Anti-IL-5 Antibodies in *in vitro* Bioassay

The activities of various CDR grafted antibodies were compared with that of chimeric 39D10 in an *in vitro* bioassay using TF1 cells. TF1 is an erythroleukemic cell line that requires GM-CSF for growth. GM-CSF can be replaced by IL-5 but in this instance the cells only survive and do not proliferate. However the dependence on IL-5 for survival means that TF1 cells can be used in a bioassay to compare the activities of various anti-IL-5 antibodies.

Neutralisation by anti-IL-5 antibodies was measured using a constant amount of IL-5 (2ng/ml) and variable amounts of antibody incubated with $5x10^4$ cells per well in 96 flat bottomed plates for 3 days. For the last 4 hours, cells are

cultured in the presence of 500 μg/ml Thiazolyl blue (MIT). This dye is converted into an insoluble purple form by mitochondrial enzymes in viable cells. The insoluble material was dissolved by incubating overnight after addition of 100 μl of 50% dimethyl formamide, 20% SDS pH4.7 and the amount of dye taken up determined spectrophotometrically. The levels of bioactive IL-5 remaining in the presence of the antibodies is extrapolated from a standard curve relating dye uptake to IL-5 concentration.

The activities of various combination of heavy and light chains were evaluated using the TF1 bioassay. The results are shown in Figure 10. It can be seen that all combinations of CDR grafted heavy and light chains produce antibodies that are equipotent with chimeric 39D10. These results indicate that neither residue 22 in the light chain nor residues 23 or 78 in the heavy chain are required to be 39D10 specific for optimal binding. The combination with the fewer 39D10 specific residues is therefore CTIL-5-10gH/-gL6.

Activity of Anti-IL-5 Antibodies in Competition Assays

Recombinant human IL-5 was diluted to 1 μg/ml in phosphate buffered saline (PBS) and 100 μl aliquots added to microtitre plates (Costar Amine Binding plates) and incubated overnight at 4°C. Plates were washed three times with PBS containing 0.5% Tween 20 and any remaining active sites blocked with 2% bovine serum albumin (BSA) in PBS for 30 minutes. The plates were then aspirated and tapped dry. To compare the relative binding activity of the parent rat antibody (39D10) with chimeric and grafted antibodies, serial dilutions were prepared of each anti-IL-5 antibody in PBS/1% BSA and 50 μl added to duplicate wells followed immediately by 50 μl 39D10-biotin conjugate at 0.125 μg/ml. The assay was incubated for 2 hours at room temperature with agitation and then washed twice with PBS. Horseradish-

peroxidase conjugated to streptavidin (1 μg/ml) was added to all wells and incubated for a further 30 minutes. Plates were washed four times and 100 μl tetramethyl benzidine (TMB) substrate added. Colour development was read at 630 nm (reference 490 nm) and OD (630-490) was plotted against log (10) antibody concentration.

When the activities of rat 39D10, chimeric 39D10 and CTIL-5-10gH/gL6 were compared in the above competition assay, the results shown in Figure 11 were obtained. All three antibodies competed equally well with biotinylated-39D10 for binding to IL-5, indicating that the CDR loops of 39D10 had been successfully transferred to the human frameworks.

Effect of Anti-IL-5 Antibody on Monkey Eosinophilia

Anti-IL-5 antibody (CTIL-5-10gH/gL6) was tested in a monkey system which models asthmatic conditions (see Mauser, P.J. *et al.*, Ann. Rev. Respir. Dis., in press). When administered, one hour before challenge with *Ascaris*, to responsive monkeys, CTIL-5-10gH/gL6 inhibits lung lavage eosinophilia 75% at a dose of 0.3 mg/kg i.v. This set of monkeys is not hyper-responsive to histamine so the effects of CTIL-5-10gH/gL6 on hyper-responsiveness could not be determined. Three months after this single dose, eosinophil accumulation in response to *Ascaris* challenge is still inhibited 75%.

In the allergic mouse, CTIL-5-10gH/gL6 inhibits pulmonary eosinophilia at 1 mg/kg i.p.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: CELLTECH LIMITED
(B) STREET: 216 BATH ROAD
(C) CITY: SLOUGH
(D) STATE: BERKSHIRE
(E) COUNTRY: UNITED KINGDOM
(F) POSTAL CODE (ZIP): SL1 4EN
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(I) TELEX: 848473

(ii) TITLE OF INVENTION: INTERLEUKIN-5 SPECIFIC RECOMBINANT ANTIBODIES

(iii) NUMBER OF SEQUENCES: 28

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCGCGCAAGC TTGCCGCCAC CATGAAGATT GTGGTTAAAC TGGGTTTT 48

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCAGATGGGC CCTTCGTTGA GGCTGACAGG AGACGTAGTG A 41

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGACTGTTCG AAGCCGCCAC CATGAGTGTG CTCACTCAGG TCCT 44

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGATACAGTT GGTGCAGCAT CCGTACGTTT 30

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..333

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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GAA TCT GGA GGA GGC TTG GTA CAG CCA TCA CAG ACC CTG TCT CTC ACC        48
Glu Ser Gly Gly Gly Leu Val Gln Pro Ser Gln Thr Leu Ser Leu Thr
  1               5                  10                  15

TGC ACT GTC TCT GGG TTA TCA TTA ACC AGC AAT AGT GTG AAC TGG ATT        96
Cys Thr Val Ser Gly Leu Ser Leu Thr Ser Asn Ser Val Asn Trp Ile
             20                  25                  30

CGG CAG CCT CCA GGA AAG GGT CTG GAG TGG ATG GGA CTA ATA TGG AGT       144
Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met Gly Leu Ile Trp Ser
         35                  40                  45

AAT GGA GAC ACA GAT TAT AAT TCA GCT ATC AAA TCC CGA CTG AGC ATC       192
Asn Gly Asp Thr Asp Tyr Asn Ser Ala Ile Lys Ser Arg Leu Ser Ile
     50                  55                  60

AGT AGG GAC ACC TCG AAG AGC CAG GTT TTC TTA AAG ATG AAC AGT CTG       240
Ser Arg Asp Thr Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu
 65                  70                  75                  80

CAA AGT GAA GAC ACA GCC ATG TAC TTC TGT GCC AGA GAG TAC TAC GGC       288
Gln Ser Glu Asp Thr Ala Met Tyr Phe Cys Ala Arg Glu Tyr Tyr Gly
                 85                  90                  95

TAC TTT GAT TAC TGG GGC CAA GGA GTC ATG GTC ACA GTC TCC TCA           333
Tyr Phe Asp Tyr Trp Gly Gln Gly Val Met Val Thr Val Ser Ser
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Glu Ser Gly Gly Gly Leu Val Gln Pro Ser Gln Thr Leu Ser Leu Thr
 1               5                  10                  15

Cys Thr Val Ser Gly Leu Ser Leu Thr Ser Asn Ser Val Asn Trp Ile
            20                  25                  30

Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met Gly Leu Ile Trp Ser
        35                  40                  45

Asn Gly Asp Thr Asp Tyr Asn Ser Ala Ile Lys Ser Arg Leu Ser Ile
    50                  55                  60

Ser Arg Asp Thr Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu
65                  70                  75                  80

Gln Ser Glu Asp Thr Ala Met Tyr Phe Cys Ala Arg Glu Tyr Tyr Gly
                85                  90                  95

Tyr Phe Asp Tyr Trp Gly Gln Gly Val Met Val Thr Val Ser Ser
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 384 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..384

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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ATG GCT GTG CCC ACT CAG CTC CTG GGG TTG TTG TTG CTG TGG ATT ACA      48
Met Ala Val Pro Thr Gln Leu Leu Gly Leu Leu Leu Leu Trp Ile Thr
 1               5                  10                  15

GAT GCC ATA TGT GAC ATC CAG ATG ACA CAG TCT CCA GCT TCC CTG TCT      96
Asp Ala Ile Cys Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser
            20                  25                  30

GCA TCT CTG GGA GAA ACT ATC TCC ATC GAA TGT CTA GCA AGT GAG GGC     144
Ala Ser Leu Gly Glu Thr Ile Ser Ile Glu Cys Leu Ala Ser Glu Gly
        35                  40                  45

ATT TCC AGT TAT TTA GCG TGG TAT CAG CAG AAG CCA GGG AAA TCT CCT     192
Ile Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro
    50                  55                  60

CAG CTC CTG ATC TAT GGT GCA AAT AGC TTG CAA ACT GGG GTC CCA TCA     240
Gln Leu Leu Ile Tyr Gly Ala Asn Ser Leu Gln Thr Gly Val Pro Ser
65                  70                  75                  80
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```
CGG TTC AGT GGC AGT GGA TCT GCC ACA CAA TAT TCT CTC AAG ATC AGC        288
Arg Phe Ser Gly Ser Gly Ser Ala Thr Gln Tyr Ser Leu Lys Ile Ser
                85                  90                  95

AGC ATG CAA CCT GAA GAT GAA GGG GAT TAT TTC TGT CAA CAG AGT TAC        336
Ser Met Gln Pro Glu Asp Glu Gly Asp Tyr Phe Cys Gln Gln Ser Tyr
            100                 105                 110

AAG TTT CCG AAC ACG TTT GGA GCT GGG ACC AAG CTG GAA CTG AAA CGG        384
Lys Phe Pro Asn Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Ala Val Pro Thr Gln Leu Leu Gly Leu Leu Leu Trp Ile Thr
 1               5                  10                  15

Asp Ala Ile Cys Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser
            20                  25                  30

Ala Ser Leu Gly Glu Thr Ile Ser Ile Glu Cys Leu Ala Ser Glu Gly
        35                  40                  45

Ile Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro
    50                  55                  60

Gln Leu Leu Ile Tyr Gly Ala Asn Ser Leu Gln Thr Gly Val Pro Ser
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Ala Thr Gln Tyr Ser Leu Lys Ile Ser
                85                  90                  95

Ser Met Gln Pro Glu Asp Glu Gly Asp Tyr Phe Cys Gln Gln Ser Tyr
            100                 105                 110

Lys Phe Pro Asn Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys
            20
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Glu Thr Ile Ser Ile Glu Cys
20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
20 25 30

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Ala Thr Gln Tyr Ser
1 5 10 15

Leu Lys Ile Ser Ser Met Gln Pro Glu Asp Glu Gly Asp Tyr Phe Cys
20 25 30

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Glu Ser Gly Gly Gly Leu Val Gln Pro Ser Gln Thr Leu Ser Leu Thr
1 5 10 15

Cys Thr Val Ser Gly Leu Ser Leu Thr
20 25

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Arg Leu Ser Ile Ser Arg Asp Thr Ser Lys Ser Gln Val Phe Leu Lys
1 5 10 15

Met Asn Ser Leu Gln Ser Glu Asp Thr Ala Met Tyr Phe Cys Ala Arg
20 25 30

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Trp Gly Gln Gly Val Met Val Thr Val Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..399

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

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TTC GAA GCC GCC ACC ATG TCT GTC CCC ACC CAA GTC CTC GGT CTC CTG          48
Phe Glu Ala Ala Thr Met Ser Val Pro Thr Gln Val Leu Gly Leu Leu
 1               5                  10                  15

CTG CTG TGG CTT ACA GAT GCC AGA TGT GAC ATT CAA ATG ACC CAG AGC          96
Leu Leu Trp Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser
            20                  25                  30

CCA TCC AGC CTG AGC GCA TCT GTA GGA GAC CGG GTC ACC ATC ACA TGT         144
Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
        35                  40                  45

CTA GCA AGT GAG GGC ATC TCC AGT TAC TTA GCG TGG TAC CAG CAG AAG         192
Leu Ala Ser Glu Gly Ile Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys
    50                  55                  60

CCC GGG AAA GCT CCT AAG CTC CTG ATC TAT GGT GCG AAT AGC TTG CAG         240
Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Gly Ala Asn Ser Leu Gln
65                  70                  75                  80

ACT GGA GTA CCA TCA AGA TTC AGT GGC TCA GGA TCC GCT ACA GAC TAC         288
Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Ala Thr Asp Tyr
                85                  90                  95

ACG CTC ACG ATC TCC AGC CTA CAG CCT GAA GAT TTC GCA ACG TAT TAC         336
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
            100                 105                 110

TGT CAA CAG TCG TAT AAG TTC CCG AAC ACA TTC GGT CAA GGC ACC AAG         384
Cys Gln Gln Ser Tyr Lys Phe Pro Asn Thr Phe Gly Gln Gly Thr Lys
        115                 120                 125

GTC GAA GTC AAA CGT                                                    399
Val Glu Val Lys Arg
    130
```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```
Phe Glu Ala Ala Thr Met Ser Val Pro Thr Gln Val Leu Gly Leu Leu
 1               5                  10                  15

Leu Leu Trp Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser
```

```
            20                  25                  30

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
        35                  40                  45

Leu Ala Ser Glu Gly Ile Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys
    50                  55                  60

Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Gly Ala Asn Ser Leu Gln
65                  70                  75                  80

Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Ala Thr Asp Tyr
                85                  90                  95

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
            100                 105                 110

Cys Gln Gln Ser Tyr Lys Phe Pro Asn Thr Phe Gly Gln Gly Thr Lys
        115                 120                 125

Val Glu Val Lys Arg
    130
```

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..420

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
AAG CTT GCC GCC ACC ATG GGC TGG AGC TGT ATC ATC CTC TTC TTA GTA      48
Lys Leu Ala Ala Thr Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val
 1               5                  10                  15

GCA ACA GCT ACA GGT GTC CAC TCC GAG GTC CAA CTG GTA GAA TCT GGA      96
Ala Thr Ala Thr Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly
            20                  25                  30

GGT GGT CTC GTA CAG CCA GGA GGA TCT CTG CGA CTG AGT TGC GCC GTC     144
Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val
        35                  40                  45

TCT GGG TTA TCA TTA ACT AGT AAT AGT GTG AAC TGG ATA CGG CAA GCA     192
Ser Gly Leu Ser Leu Thr Ser Asn Ser Val Asn Trp Ile Arg Gln Ala
    50                  55                  60

CCT GGC AAG GGT CTC GAG TGG GTT GGA CTA ATA TGG AGT AAT GGA GAC     240
Pro Gly Lys Gly Leu Glu Trp Val Gly Leu Ile Trp Ser Asn Gly Asp
65                  70                  75                  80

ACA GAT TAT AAT TCA GCT ATC AAA TCT CGA TTC ACA ATC TCT AGA GAC     288
Thr Asp Tyr Asn Ser Ala Ile Lys Ser Arg Phe Thr Ile Ser Arg Asp
                85                  90                  95

ACT TCG AAG AGC ACC GTA TAC CTG CAG ATG AAC AGT CTG AGA GCT GAA     336
Thr Ser Lys Ser Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu
```

```
          100                 105                 110
GAT ACT GCA GTC TAC TAC TGT GCT CGT GAG TAC TAT GGA TAT TTC GAC      384
Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Gly Tyr Phe Asp
        115                 120                 125

TAT TGG GGT CAA GGT ACC CTA GTC ACA GTC TCC TCA                      420
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
    130                 135                 140
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

```
Lys Leu Ala Ala Thr Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val
 1               5                  10                  15

Ala Thr Ala Thr Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly
            20                  25                  30

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val
        35                  40                  45

Ser Gly Leu Ser Leu Thr Ser Asn Ser Val Asn Trp Ile Arg Gln Ala
    50                  55                  60

Pro Gly Lys Gly Leu Glu Trp Val Gly Leu Ile Trp Ser Asn Gly Asp
65                  70                  75                  80

Thr Asp Tyr Asn Ser Ala Ile Lys Ser Arg Phe Thr Ile Ser Arg Asp
                85                  90                  95

Thr Ser Lys Ser Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu
            100                 105                 110

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Gly Tyr Phe Asp
        115                 120                 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
    130                 135                 140
```

CLAIMS

1. A RAM having affinity for human IL-5 antigen and comprising antigen binding regions derived from heavy and/or light chain variable domains of a donor antibody having affinity for human IL-5, the RAM having a binding affinity similar to that of the donor antibody.

2. The anti-IL-5 antibody molecule of claim 1 comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising predominately acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for human IL-5, wherein, said composite heavy chain comprises donor residues at least at positions 31-35, 50-65 and 95-102 (according to the Kabat numbering system).

3. The antibody molecule of claim 2, wherein, amino acid residues 24, 27-30, 37, 49, 73, 76 and 78 in said composite heavy chain are additionally donor residues.

4. The antibody molecule of claim 2, wherein amino acid residues 23, 24, 27-30, 37, 49, 73 and 76-78 in said composite heavy chain are additionally donor residues.

5. The anti-IL-5 antibody molecule of claim 1 comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising predominately acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for human IL-5, wherein, said composite light chain comprises donor residues at least at positions 24-34, 50-56 and 89-97 (according to the Kabat numbering system) and wherein, said anti-IL-5 antibody molecule has a binding affinity similar to that of the donor antibody.

6. The antibody molecule of claim 5 wherein amino acid

residues 68 and 71 in said composite light chain are additionally donor residues.

7. The antibody molecule of claim 5 wherein amino acid residues 22, 68 and 71 in said composite light chain are additionally donor residues.

8. An anti-IL-5 antibody molecule having affinity for human IL-5 compressing the composite heavy chain of any one of claims 2 to 4 and the composite light chain of any one of claims 5 to 7.

9. The antibody molecule of any one of claims 2 to 8 comprising predominantly human acceptor residues and non-human donor residues.

10. The antibody molecule of any one of claims 2 to 9 wherein the acceptor residues for the composite heavy and light chains are human group III heavy chain and human group I light chain residues respectively, and the donor residues for the composite heavy and light chains are rat 39D10 heavy and light chain residues respectively.

11. A DNA sequence which encodes for the composite heavy chain or the composite light chain of an antibody according to any one of claims 2 to 10.

12. A cloning or expression vector containing a DNA sequence according to claim 11.

13. A host cell transformed with a DNA sequence according to claim 11.

14. A process for the production of an anti-IL-5 antibody comprising expressing at least one DNA sequence according to claim 11 in a transformed host cell.

15. A process for producing an anti-IL-5 antibody molecule

comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes a composite heavy chain according to any one of claims 2 to 4;

(b) optionally producing in an expression vector an operon having a DNA sequence which encodes a complementary light chain which may be a composite light chain according to any one of claims 5 to 7;

(c) transvecting a host cell with the or each vector;

and

(d) culturing the transvected cell line to produce the antibody product.

16. A process for producing an anti-IL-5 antibody molecule according to claim 15 wherein the DNA sequences encode a composite light chain and a complementary heavy chain, respectively.

17. A therapeutic or diagnostic composition comprising the antibody molecule according to any one of claims 1 to 10 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

18. A method of therapy or diagnosis comprising administering an effective amount of an antibody molecule according to any one of claims 1 to 10 to a human or animal subject.

FIG.1

```
GAA TCT GGA GGA GGC TTG GTA CAG CCA TCA CAG ACC CTG TCT CTC
 E   S   G   G   G   L   V   Q   P   S   Q   T   L   S   L>

ACC TGC ACT GTC TCT GGG TTA TCA TTA ACC AGC AAT AGT GTG AAC
 T   C   T   V   S   G   L   S   L   T   S   N   S   V   N>

TGG ATT CGG CAG CCT CCA GGA AAG GGT CTG GAG TGG ATG GGA CTA
 W   I   R   Q   P   P   G   K   G   L   E   W   M   G   L>

ATA TGG AGT AAT GGA GAC ACA GAT TAT AAT TCA GCT ATC AAA TCC
 I   W   S   N   G   D   T   D   Y   N   S   A   I   K   S>

CGA CTG AGC ATC AGT AGG GAC ACC TCG AAG AGC CAG GTT TTC TTA
 R   L   S   I   S   R   D   T   S   K   S   Q   V   F   L

AAG ATG AAC AGT CTG CAA AGT GAA GAC ACA GCC ATG TAC TTC TGT
 K   M   N   S   L   Q   S   E   D   T   A   M   Y   F   C>

GCC AGA GAG TAC TAC GGC TAC TTT GAT TAC TGG GGC CAA GGA GTC
 A   R   E   Y   Y   G   Y   F   D   Y   W   G   Q   G   V

ATG GTC ACA GTC TCC TCA
 M   V   T   V   S   S>
```

FIG. 2

```
ATG GCT GTG CCC ACT CAG CTC CTG GGG TTG TTG TTG CTG TGG ATT
 M   A   V   P   T   Q   L   L   G   L   L   L   L   W   I>

ACA GAT GCC ATA TGT GAC ATC CAG ATG ACA CAG TCT CCA GCT TCC
 T   D   A   I   C   D   I   Q   M   T   Q   S   P   A   S>

CTG TCT GCA TCT CTG GGA GAA ACT ATC TCC ATC GAA TGT CTA GCA
 L   S   A   S   L   G   E   T   I   S   I   E   C   L   A>

AGT GAG GGC ATT TCC AGT TAT TTA GCG TGG TAT CAG CAG AAG CCA
 S   E   G   I   S   S   Y   L   A   W   Y   Q   Q   K   P>

GGG AAA TCT CCT CAG CTC CTG ATC TAT GGT GCA AAT AGC TTG CAA
 G   K   S   P   Q   L   L   I   Y   G   A   N   S   L   Q>

ACT GGG GTC CCA TCA CGG TTC AGT GGC AGT GGA TCT GCC ACA CAA
 T   G   V   P   S   R   F   S   G   S   G   S   A   T   Q

TAT TCT CTC AAG ATC AGC AGC ATG CAA CCT GAA GAT GAA GGG GAT
 Y   S   L   K   I   S   S   M   Q   P   E   D   E   G   D>

TAT TTC TGT CAA CAG AGT TAC AAG TTT CCG AAC ACG TTT GGA GCT
 Y   F   C   Q   Q   S   Y   K   F   P   N   T   F   G   A>

GGG ACC AAG CTG GAA CTG AAA CGG
 G   T   K   L   E   L   K   R>
```

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FIG. 3

Framework 1

```
                                  1 1112 2
                              9   5 7890 2
        hu gp1   DIQMTQSPSSLSASVGDRVTITC
        39D10    DIQMTQSPASLSASLGETISIEC
```

Framework 2

```
                              4 4
                              3 5
        hu gp1   WYQQKPGKAPKLLIY
        39D10    WYQQKPGKSPQLLIY
```

Framework 3

```
                                  6 777 7   7     888 8
                                  8 012 4   8     345 7
        hu gp1   GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
        39d10    GVPSRFSGSGSATQYSLKISSMQPEDEGDYFC
```

Framework 4

```
                         1   1 1
                         0   0 0
                         0   4 6
        hu gp1   FGQGTKVEIKR
        39D10    FGAGTKLELKR
```

FIG. 4

Framework 1

```
                                    111 1 2 22  2223
                                    567 9 1 34  7890
          hu gp3   EVQLVESGGGLVQPGGSLRLSCAASGFTFS
          39D10    ?????ESGGGLVQPSQTLSLTCTVSGLSLT
```

Framework 2

```
                     3  4       44
                     7  0       89
          hu gp3   WVRQAPGKGLEWVS
          39D10    WIRQPPGKGLEWMG
```

Framework 3

```
                      66    7  7777 8    88    8 9
                      78    3  6789 1    34    9 1
          hu gp3   RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
          39D10    RLSISRDTSKSQVFLKMNSLQSEDTAMYFCAR
```

Framework 4

```
                        11
                        00
                        78
          hu gp3   WGQGTLVTVSS
          39D10    WGQGVMVTVSS
```

FIG. 5

```
TTCGAAGCCGCCACC ATG TCT GTC CCC ACC CAA GTC CTC GGT CTC CTG
                  M   S   V   P   T   Q   V   L   G   L   L>

CTG CTG TGG CTT ACA GAT GCC AGA TGT GAC ATT CAA ATG ACC CAG
 L   L   W   L   T   D   A   R   C   D   I   Q   M   T   Q>

AGC CCA TCC AGC CTG AGC GCA TCT GTA GGA GAC CGG GTC ACC ATC
 S   P   S   S   L   S   A   S   V   G   D   R   V   T   I>

ACA TGT CTA GCA AGT GAG GGC ATC TCC AGT TAC TTA GCG TGG TAC
 T   C   L   A   S   E   G   I   S   S   Y   L   A   W   Y>

CAG CAG AAG CCC GGG AAA GCT CCT AAG CTC CTG ATC TAT GGT GCG
 Q   Q   K   P   G   K   A   P   K   L   L   I   Y   G   A>

AAT AGC TTG CAG ACT GGA GTA CCA TCA AGA TTC AGT GGC TCA GGA
 N   S   L   Q   T   G   V   P   S   R   F   S   G   S   G>

TCC GCT ACA GAC TAC ACG CTC ACG ATC TCC AGC CTA CAG CCT GAA
 S   A   T   D   Y   T   L   T   I   S   S   L   Q   P   E>

GAT TTC GCA ACG TAT TAC TGT CAA CAG TCG TAT AAG TTC CCG AAC
 D   F   A   T   Y   Y   C   Q   Q   S   Y   K   F   P   N>

ACA TTC GGT CAA GGC ACC AAG GTC GAA GTC AAA CGT
 T   F   G   Q   G   T   K   V   E   V   K   R>
```

FIG. 6

```
AAGCTTGCCGCCACC ATG GGC TGG AGC TGT ATC ATC CTC TTC TTA GTA
                  M   G   W   S   C   I   I   L   F   L   V>

GCA ACA GCT ACA GGT GTC CAC TCC GAG GTC CAA CTG GTA GAA TCT
 A   T   A   T   G   V   H   S   E   V   Q   L   V   E   S>

GGA GGT GGT CTC GTA CAG CCA GGA GGA TCT CTG CGA CTG AGT TGC
 G   G   G   L   V   Q   P   G   G   S   L   R   L   S   C>

GCC GTC TCT GGG TTA TCA TTA ACT AGT AAT AGT GTG AAC TGG ATA
 A   V   S   G   L   S   L   T   S   N   S   V   N   W   I>

CGG CAA GCA CCT GGC AAG GGT CTC GAG TGG GTT GGA CTA ATA TGG
 R   Q   A   P   G   K   G   L   E   W   V   G   L   I   W>

AGT AAT GGA GAC ACA GAT TAT AAT TCA GCT ATC AAA TCT CGA TTC
 S   N   G   D   T   D   Y   N   S   A   I   K   S   R   F>

ACA ATC TCT AGA GAC ACT TCG AAG AGC ACC GTA TAC CTG CAG ATG
 T   I   S   R   D   T   S   K   S   T   V   Y   L   Q   M>

AAC AGT CTG AGA GCT GAA GAT ACT GCA GTC TAC TAC TGT GCT CGT
 N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   R>

GAG TAC TAT GGA TAT TTC GAC TAT TGG GGT CAA GGT ACC CTA GTC
 E   Y   Y   G   Y   F   D   Y   W   G   Q   G   T   L   V>

ACA GTC TCC TCA
 T   V   S   S>
```

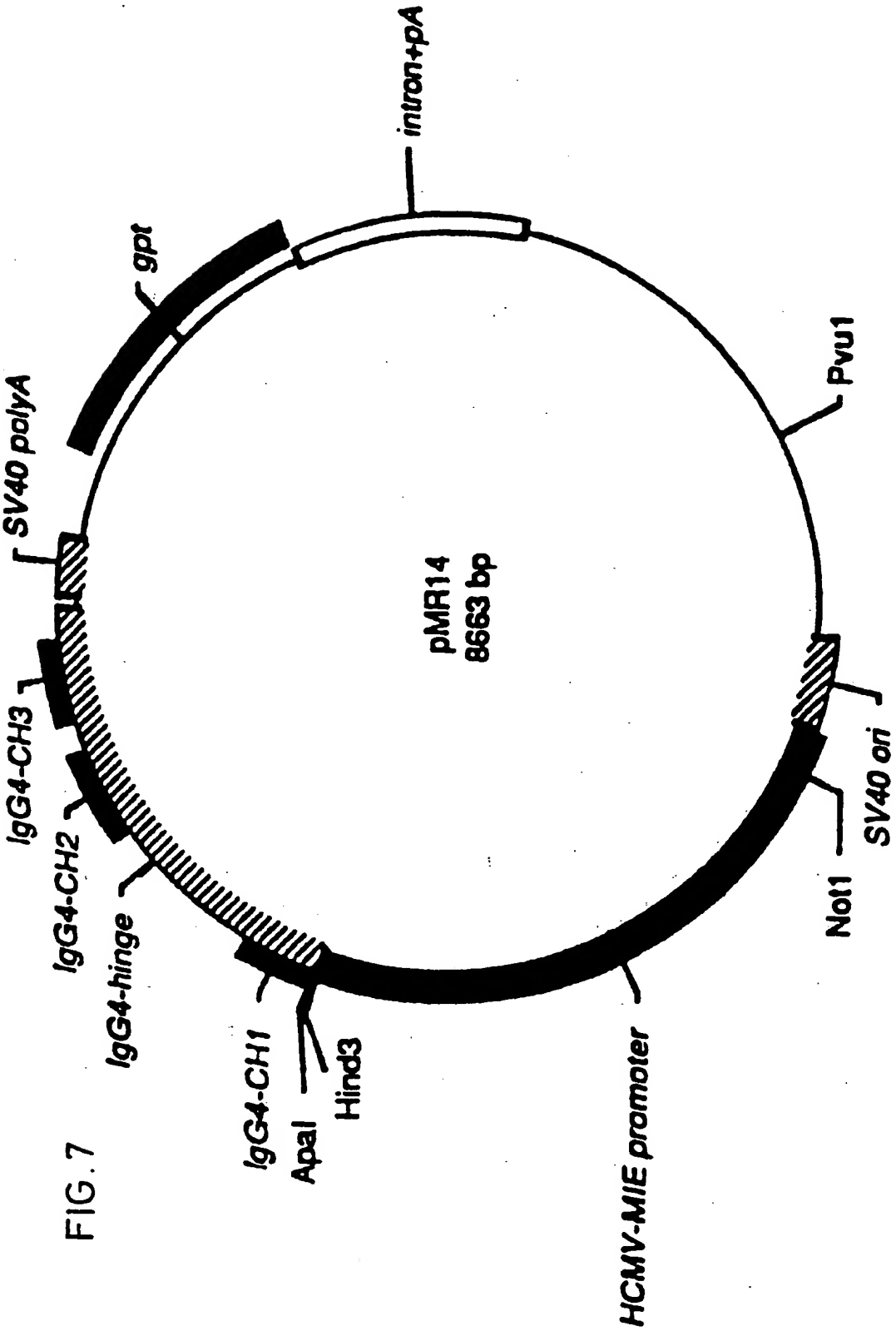

intron+pA
gpt
SV40 polyA
Pvu1
pMR14
8663 bp
IgG4-CH3
IgG4-CH2
IgG4-hinge
IgG4-CH1
ApaI
Hind3
HCMV-MIE promoter
Not1
SV40 ori

FIG. 7

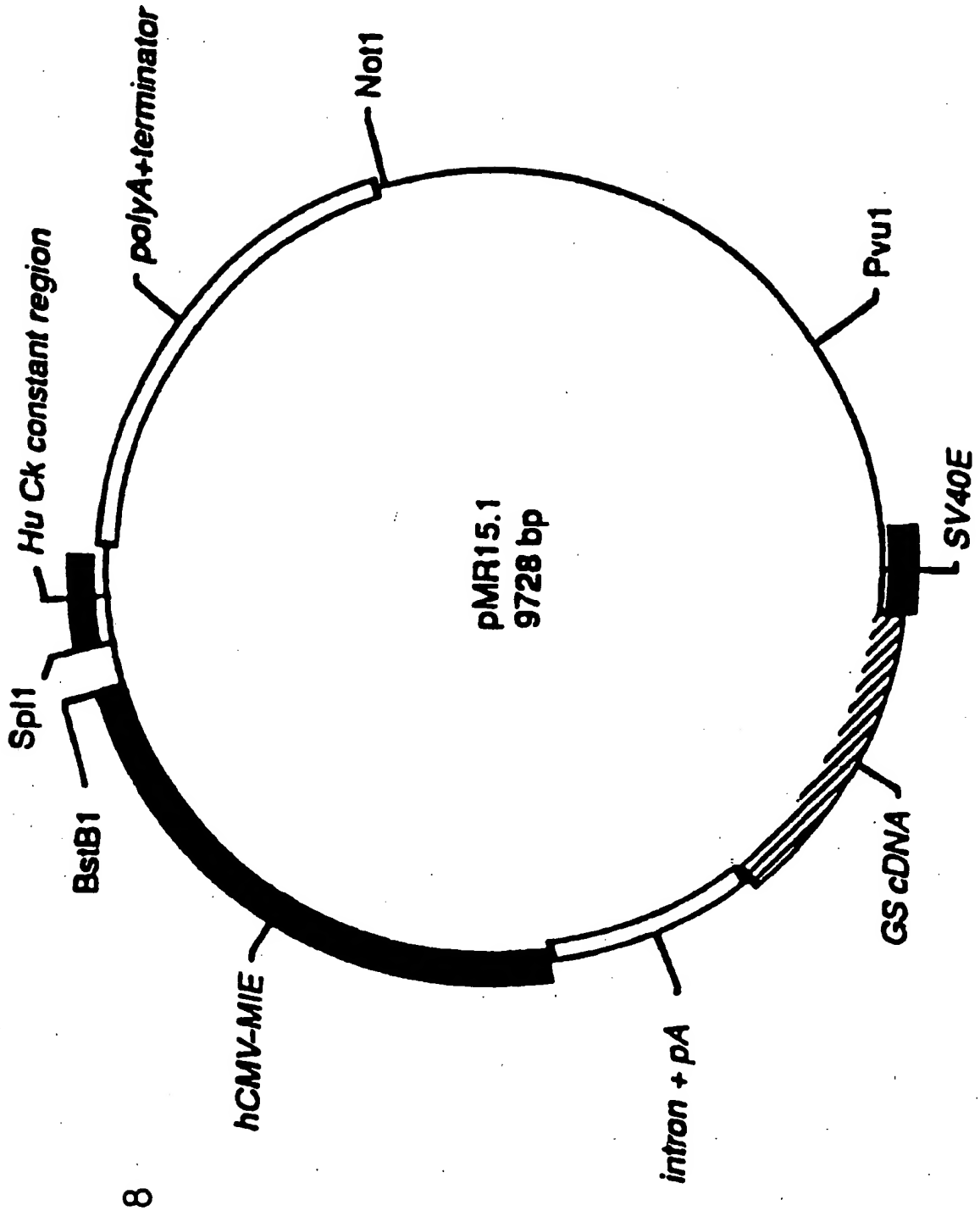

polyA+terminator
Not1
Hu Ck constant region
Pvu1
pMR15.1
9728 bp
SV40E
Spl1
BstB1
GS cDNA
hCMV-MIE
intron + pA

FIG.8

FIG. 9

Antibody	kd ($x10^{-10}M$)	kass ($x10^{5}M^{-1}sec^{-1}$)
Chimeric 39D10	1.14	5.77
	0.63	6.27
CTIL-5-10gH/gL6	0.89	4.55
	1.18	4.72

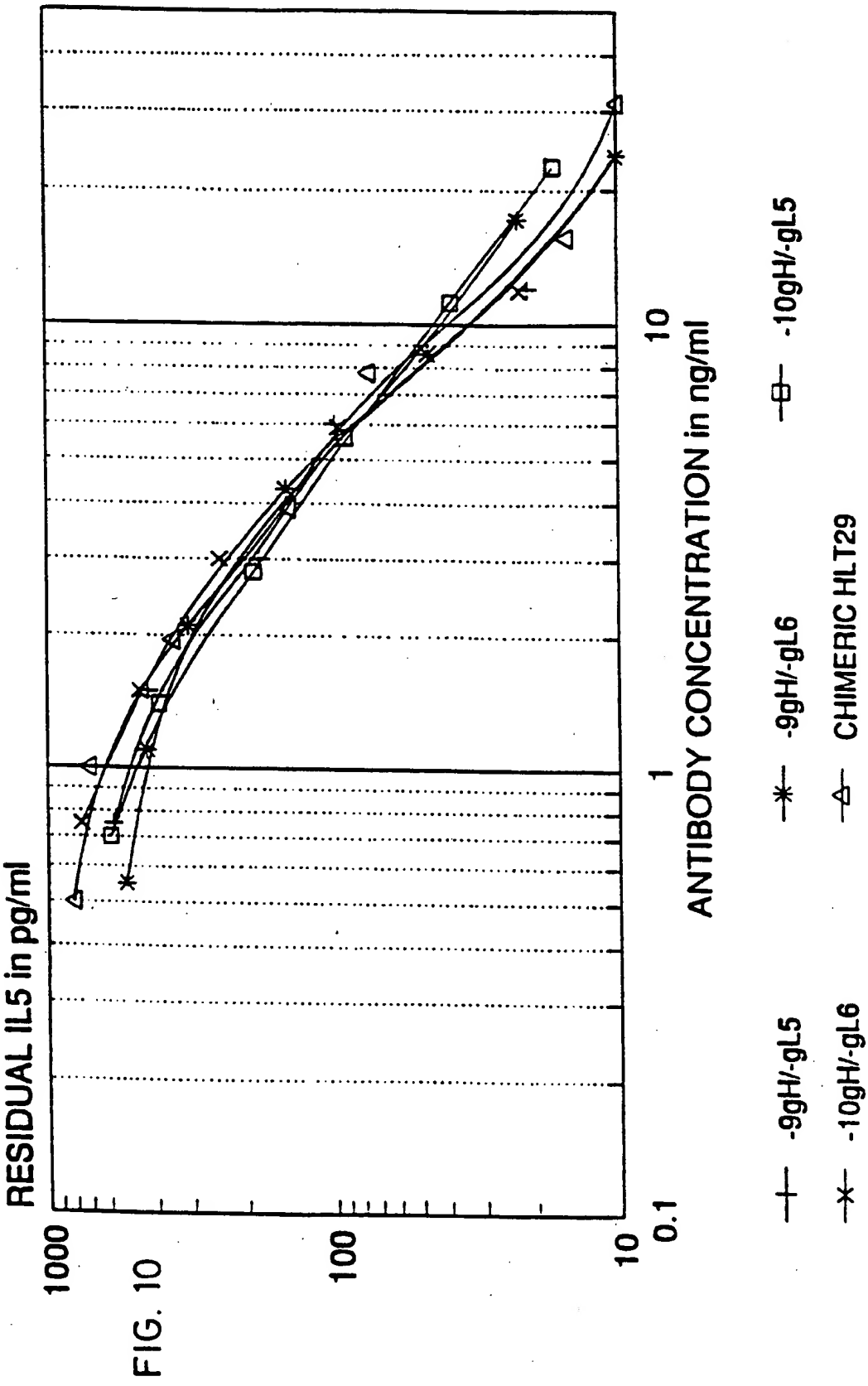
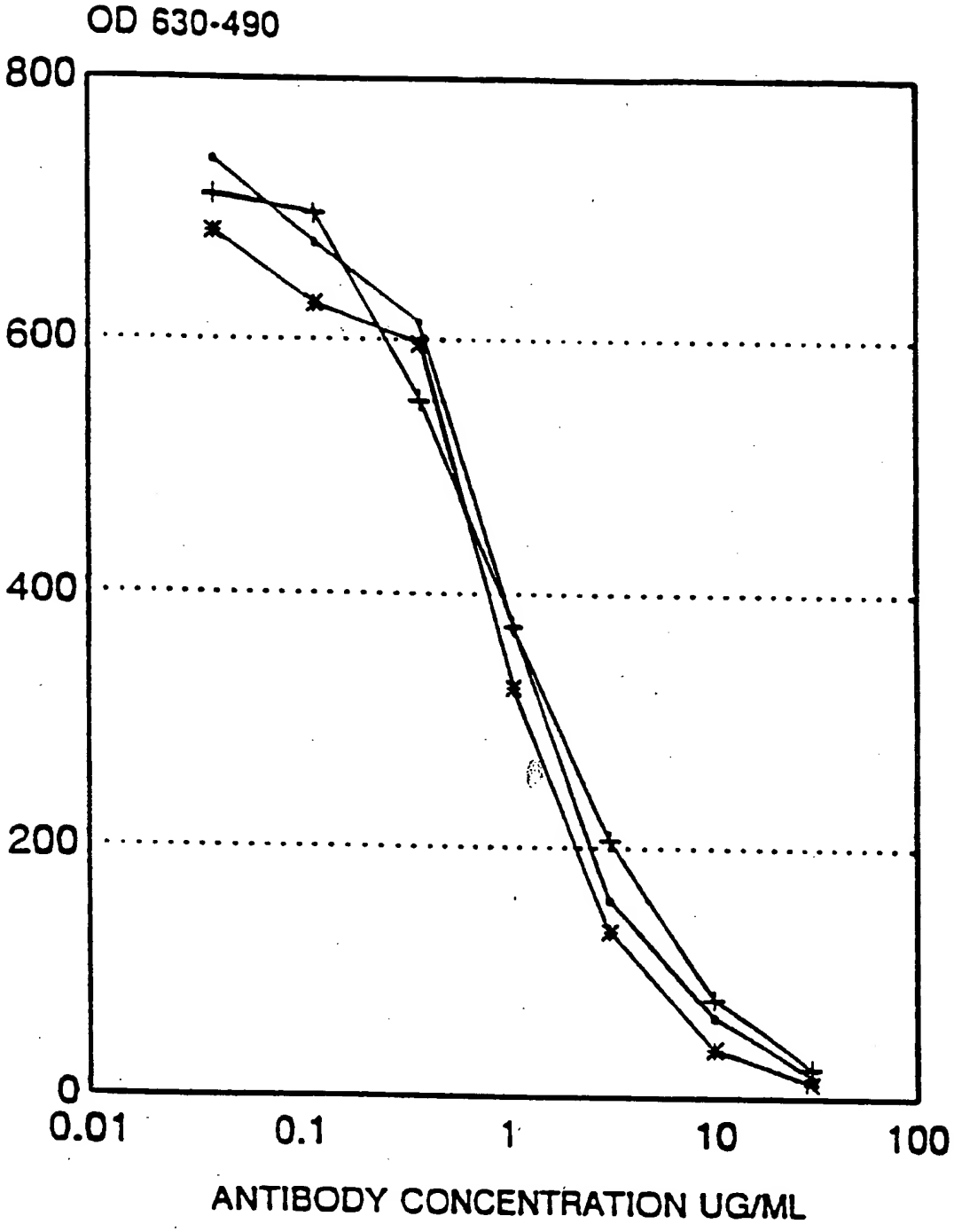

RESIDUAL IL5 in pg/ml
1000
100
10
0.1
1
10
ANTIBODY CONCENTRATION in ng/ml
-10gH/-gL5
-9gH/-gL6
CHIMERIC HLT29
-9gH/-gL5
-10gH/-gL6

FIG. 10

FIG.11


OD 630-490
800
600
400
200
0
0.01
0.1
1
10
100
ANTIBODY CONCENTRATION UG/ML

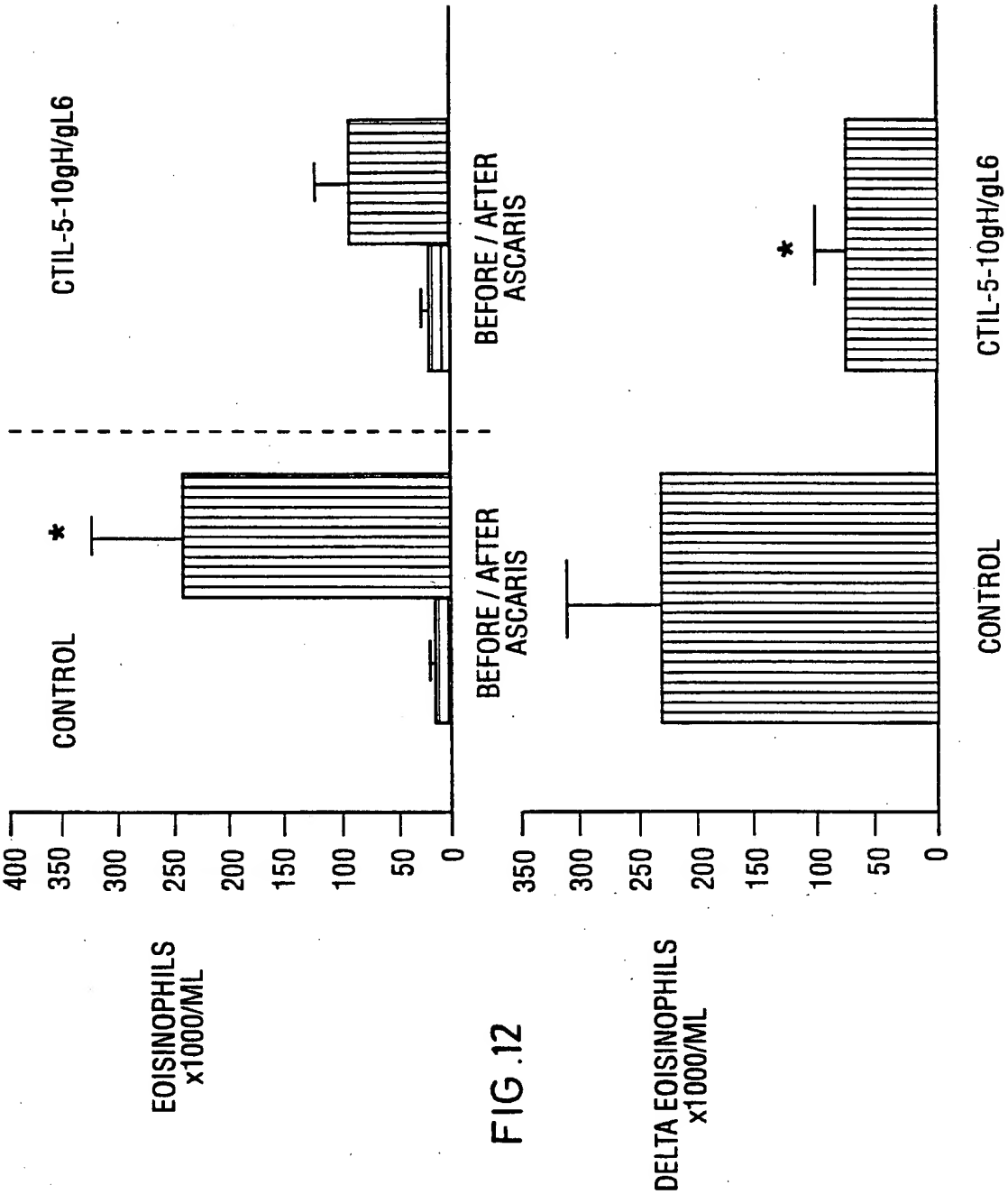

EOISINOPHILS x1000/ML
400
350
300
250
200
150
100
50
0
CONTROL
*
BEFORE / AFTER ASCARIS
CTIL-5-10gH/gL6
BEFORE / AFTER ASCARIS
DELTA EOISINOPHILS x1000/ML
350
300
250
200
150
100
50
0
CONTROL
*
CTIL-5-10gH/gL6

FIG .12

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 95/01411

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/24 C12N15/85 C12N5/10 G01N33/577
A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 16184 (SCHERING CORPORATION) 19 August 1993 cited in the application see the whole document ---	1-18
Y	WO,A,91 09967 (CELLTECH LIMITED) 11 July 1991 cited in the application see examples see claims --- -/--	1-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 1995	09. 11. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 95/01411

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BLOOD, vol. 77, no. 7, 1 April 1991 NEW YORK, NY, USA, pages 1462-1468, J. DENBURG ET AL. 'Interleukin-5 is a human basophilopoietin: Induction of histamine content and basophilic differentiation of HL-60 cells and of peripheral blood basophil-eosinophil progenitors.' see the whole document	1-18
A	WO,A,92 08474 (THE NATIONAL HEART AND LUNG INSTITUTE) 29 May 1992 see page 21, line 4 - line 12 see page 27, line 26 - page 28, line 2 see claims	1-18
A	WO,A,93 17106 (SCHERING CORPORATION) 2 September 1993 see the whole document	1-18
A	EP,A,0 367 596 (SCHERING CORPORATION) 9 May 1990 see the whole document	1-18

INTERNATIONAL SEARCH REPORT

In. ational application No.
PCT/GB95/01411

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Interm al Application No
PCT/GB 95/01411

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9316184	19-08-93	AU-B-	3656093	03-09-93
		CA-A-	2129445	07-08-93
		CZ-A-	9401910	15-12-94
		EP-A-	0625201	23-11-94
		FI-A-	943635	05-08-94
		HU-A-	67943	29-05-95
		JP-T-	7505767	29-06-95
		NO-A-	942912	06-10-94
		ZA-A-	9300779	05-08-93
WO-A-9109967	11-07-91	AT-T-	124459	15-07-95
		AU-B-	6461294	22-12-94
		AU-B-	646009	03-02-94
		AU-B-	6974091	24-07-91
		AU-B-	649645	02-06-94
		AU-B-	7033091	24-07-91
		AU-B-	631481	26-11-92
		AU-B-	7048691	24-07-91
		BG-B-	60462	28-04-95
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		EP-A-	0460167	11-12-91
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		EP-A-	0460178	11-12-91
		EP-A-	0620276	19-10-94
		EP-A-	0626390	30-11-94
		WO-A-	9109966	11-07-91
		WO-A-	9109968	11-07-91
		GB-A,B	2246781	12-02-92
		GB-A,B	2246570	05-02-92
		GB-A,B	2268744	19-01-94
		GB-A,B	2268745	19-01-94
		JP-T-	4505398	24-09-92
		JP-T-	4506458	12-11-92
		JP-T-	5500312	28-01-93
WO-A-9208474	29-05-92	AU-B-	8910891	11-06-92
WO-A-9317106	02-09-93	AU-B-	3666293	13-09-93
		CA-A-	2130436	02-09-93
		EP-A-	0627002	07-12-94

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 95/01411

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9317106		JP-T-	7502901	30-03-95
		ZA-A-	9301148	18-08-93
EP-A-367596	09-05-90	AU-B-	633034	21-01-93
		AU-B-	4644889	28-05-90
		CA-A-	2002144	03-05-90
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		DE-T-	68911653	07-04-94
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